Scientific Opinion on Flavouring Group Evaluation 401 (FGE.401): -Glutamyl-valyl-glycine from chemical group 34

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SCIENTIFIC OPINION

Scientific Opinion on Flavouring Group Evaluation 401 (FGE.401): γ-Glutamyl-valyl-glycine from chemical group 34[1]

EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF)[2,3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids of the European Food Safety Authority was requested to carry out a safety assessment of one flavouring substance, γ-glutamyl-valyl-glycine [FL-no: 17.038], in the Flavouring Group Evaluation 401 (FGE.401), in accordance with the Commission Regulation (EC) No 1331/2008. There is no safety concern with respect to genotoxicity for the flavouring substance. It has been demonstrated that the flavouring substance, which is a tripeptide, will be hydrolysed to the three amino acids *L*-glutamic acid, *L*-valine and glycine. As the human consumption of these three endogenous amino acids through food is orders of magnitude higher than the anticipated levels of exposure from their use as flavouring substances, the Panel concluded that γ-glutamyl-valyl-glycine [FL-no: 17.038] would be of no safety concern at its estimated level of intake as flavouring substance. The specifications for γ-glutamyl-valyl-glycine [FL-no: 17.038] are considered adequate according to Commission Regulation (EC) no 1334/2008.

KEYWORDS

food safety, flavouring, γ-glutamyl-valyl-glycine, [FL-no: 17.038], CASrn 38837-70-6

[1] On request from the Commission, Question No EFSA-Q-2013-00409 adopted on 27 March 2014.

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[3] The Panel wishes to thank the members of the Working Group on Flavourings: Ulla Beckman Sundh, Leon Brimer, Angelo Carere, Karl-Heinz Engel, Henrik Frandsen, Rainer Gürtler, Frances Hill, Trine Husøy, Wim Mennes, Gerard Mulder and Harriet Wallin for the preparatory work on this scientific opinion and the hearing experts: Vibe Beltoft, Pia Lund and Karin Nørby and EFSA's staff member : Maria Carfi, Annamaria Rossi and Kim Rygaard Nielsen for the support provided to this scientific opinion.

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SUMMARY

Following a request from the European Commission, the European Food Safety Authority (EFSA) asked the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF Panel) to deliver a scientific opinion to the Commission on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member States. In particular, EFSA was requested to carry out a safety assessment of one flavouring substance (candidate substance) in the Flavouring Group Evaluation 401 (FGE.401) in accordance with Commission Regulation (EC) no. 1334/2008.

The candidate substance, γ-glutamyl-valyl-glycine [FL-no: 17.038], is a synthetic substance; however, it has been identified as a natural component in vegetable and animal materials and has been quantitated in some unprocessed and processed food.

According to the applicant, γ-glutamyl-valyl-glycine [FL-no: 17.038] is intended to be used to modify and balance the total flavour bringing out the middle to afternote and adds brothy, creamy flavours similar to that of well-cooked stew.

The dietary exposure to the candidate substance from use as a flavouring substance is calculated to be 92 μg/kg bw/day in a 60 kg adult and 231 μg/kg bw/day in a 15 kg three-year old child. The Panel is aware that the flavouring substance is not intended to be used in the food categories specifically intended for infants and young children, however, they may also consume food from the general food categories flavoured with the candidate substance. Nevertheless, as the safety assessment of the substance is based on exposure to the separate amino acids, the Panel considers this to be of no safety concern.

There was no indication of a genotoxic potential of the candidate substance in the bacteria reversion mutation assays, in an *in vitro* chromosome aberration assay or in an *in vivo* micronucleus test.

Since it can be assumed that the candidate substance γ-glutamyl-valyl-glycine [FL-no: 17.038] is rapidly hydrolysed to its individual amino acids after consumption, the Panel based its risk assessment on the hydrolysis products, the three individual amino acids, *L*-glutamic acid (evaluated by the JECFA, JECFA no 1420), *L*-valine [FL-no: 17.028] (evaluated by the EFSA in FGE.26Rev1) and glycine [FL-no: 17.034] (evaluated by the JECFA, JECFA no 1421 and considered by EFSA in FGE.79). The human exposure to these three endogenous amino acids through food is orders of magnitude higher than the anticipated levels of exposure from their use as flavouring substances. Therefore, these three substances were not taken through the Procedure and the Panel concluded that the substances were not of safety concern at their estimated levels of intake as flavouring substances.

The specifications for [FL-no: 17.038] are considered adequate according to Commission Regulation (EC) no 1334/2008.

Based on the above considerations, the candidate substance γ-glutamyl-valyl-glycine [FL-no: 17.038] is not considered to be of safety concern at its estimated level of intake as flavouring substance.

TABLE OF CONTENTS

BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The use of flavourings is regulated under Regulation (EC) No 1334/2008[4] of the European Parliament and Council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods. On the basis of article 9(a) of this Regulation, an evaluation and approval are required for flavouring substances.

Regulation (EC) No 1331/2008 shall apply for the evaluation and approval of flavouring substances.

The Commission has received from the company Aijnomoto Co.Inc. an application from the authorisation of a new flavouring substance.

In order for the Commission to be able to consider its inclusion in the Union list of flavourings and source materials (Annex I of Regulation (EC) No 1334/2008), EFSA should carry out a safety assessment of this substance.

TERMS OF REFERENCE AS PROVIDED BY THE COMMISSION

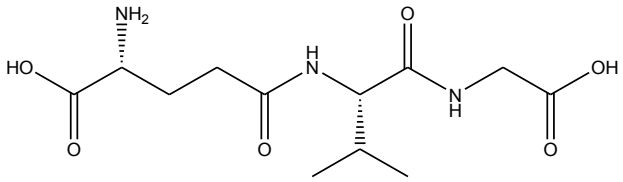
The European Commission requests the European Food Safety Authority EFSA to carry out a safety assessment on γ-glutamyl-valyl-glycine as a flavouring substance in accordance with Regulation (EC) No 1331/2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings.

[4] Regulation (EC) No 1334/2008 of the European Parliament and of the Council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods and amending Council Regulation (EEC) No 1601/91, Regulations (EC) No 2232/96 and (EC) No 110/2008 and Directive 2000/13/EC. OJ L 354, 31.12.2008, p. 34-50.

ASSESSMENT

1. Identification of the Substance

The name of the flavouring substance (candidate substance) is γ-glutamyl-valyl-glycine, FLAVIS number [FL-no: 17.038], CAS number 38837-70-6.



2. Existing Authorisations and Evaluations

The candidate substance γ-glutamyl-valyl-glycine [FL-no: 17.038] has been evaluated by the JECFA (as a structural class I compound (Cramer et al, 1978) with the JECFA no 2123) to be of no safety concern [at current estimated dietary exposure] using an NOAEL level of a JECFA-approved dipeptide as support (JECFA, 2012) and has FEMA GRAS status (FEMA 4709). The substance has not been in use previously in the European Union.

3. Manufacturing Process

3.1. Source Material

The candidate substance is chemically synthesised. Information on the source materials (starting chemicals, intermediates, reagents and process solvents) has been provided. This information has been classified as confidential by the applicant, but is available to the EFSA.

3.1.1. Genetically Modified Organism

The candidate substance is not produced by or from genetically modified organisms (GMOs).

3.2. Production Process

The principles of the synthesis have been provided. Information on the production process is confidential, but is available to the EFSA.

4. Physical/chemical properties and specifications

4.1. Chemical Name

IUPAC name: L-γ-glutamyl-L-valyl-glycine

CAS name: Glycine, L-γ-glutamyl-L-valyl-.

4.2. Identification Numbers

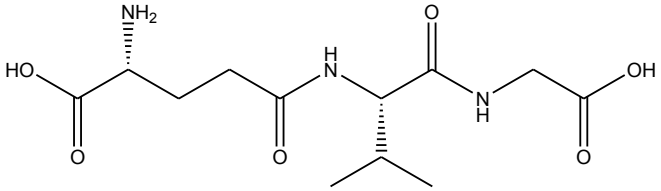
CAS-number: 38837-70-6

FLAVIS-number: [FL-no: 17.038]

JECFA-number: 2123

FEMA GRAS: 4709.

4.3. Chemical and Structural Formula, Molecular Weight



Chemical formula: $C_{12}H_{21}N_3O_6$

Molecular weight: 303.31Da.

4.4. Physical Form

Off-white powder.

4.5. Organoleptic characteristics

Odourless.

4.6. Solubility Data

Water: very soluble: 133 g/l

Ethanol: slightly soluble

Insoluble in non-polar solvents.

4.7. Identity Analysis

IR, UV, ^{1}H-and ^{13}C NMR, MS.

4.8. Purity/Minimum Assay Value

Purity of commercial preparation is > 95 % (Flavour Industry, 2014).

4.9. Impurities

The main impurities found using HPLC analysis were *L*-γ-Glutamyl-*L*-Valyl-*L*-Valyl-Glycine (2.0 %) along with *L*-α-glutamyl-*L*-valyl-glycine (α-Glu-Val-Gly) plus its cyclisation product 5-oxo-*L*-prolyl-*L*-valyl-glycine (PCA-Val-Gly) (the two together amounting to 0.7 %). Other, related small peptides were detected and the sum of all peptide impurities was about 4 %. Residual solvents were tested for by GC and the results were ethyl acetate < 50 mg/kg, methanol < 30 mg/kg, tetrahydrofuran < 10 mg/kg and toluene < 10 mg/kg (Flavour Industry, 2014).

4.10. Physical Parameters

Melting point: 225 - 228 °C.

4.11. Configuration

The candidate substance has two asymmetrical centres. The configuration is (*S*)-γ-glutamyl-(*S*)-valyl-glycine.

4.12. Stability and Decomposition Products

A 12 months stability study was conducted under 25 ˚C / 60 % RH and 40 ˚C / 75 % RH conditions. No changes were observed regarding content and enantiomeric composition; however, α-Glu-Val-Gly,

one of the major impurities (see Section 4.9) was cyclised into PCA-Val-Gly (another of the impurities found in the original commercial preparations) in a temperature and time dependent manner. The applicant proposed the expiration period to be two years for the material of commerce.

4.13. Interaction with Food Components

This was not considered by the applicant.

4.14. Particle Size

The particle size is 1 - 100 μm.

CONCLUSION ON SPECIFICATIONS

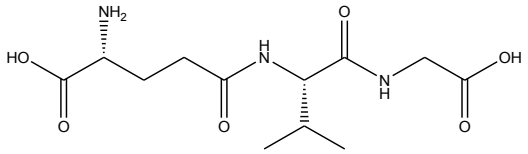
The information provided on composition and specification of the flavouring substance is considered sufficient by the Panel.

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 401

FL-no	EU Register name	Structural formula	JECFA no FEMA no CoE no CAS no EINECS no	Phys.form Mol.formula Mol.weight	Impurities	Solubility[(a)] Solubility in ethanol[(b)] Others	Boiling point, °C[(c)] Melting point, °C ID test Assay minimum	Refrac. Index[(d)] Spec.gravity[(e)]	Specification comments
17.038	γ-Glutamyl-valyl-glycine	*	2123 4709 - 38837-70-6 -	Solid $C_{12}H_{21}N_3O_6$ 303.31	5-oxo-*L*-prolyl-*L*-valyl-glycine (PCA-Val-Gly) and *L*-α-glutamyl-*L*-valyl-glycine (α-Glu-Val-Gly) 0.7 % *L*-γ-glutamyl-*L*-valyl-*L*-valyl-glycine 2.0 % Residual solvents: ethyl acetate < 50 mg/kg, methanol < 30 mg/kg, tetrahydrofuran < 10 mg/kg and toluene < 10 mg/kg	Very soluble Slightly soluble -	- 225-228 °C IR, UV, NMR, MS > 95 %	n.a. n.a.	

(a) Solubility in water, if not otherwise stated.
(b) Solubility in 95 % ethanol, if not otherwise stated.
(c) At 1013.25 hPa, if not otherwise stated.
(d) At 20°C, if not otherwise stated.
(e) At 25°C, if not otherwise stated.

*



5. Structural/Metabolic Similarity to Substances in an Existing FGE (Appendix B)

The candidate substance γ-glutamyl-valyl-glycine [FL-no: 17.038] has a number of structural elements common to chemical group 34 listed in Annex I to Commission Regulation (EC) No 1565/2000[5].

Chemical group 34 contains the amino acids. Two EFSA opinions, FGE.26Rev1 (EFSA, 2008a) and FGE.79 (EFSA, 2008b) consider the amino acids present in the Union list. Based on the results of *in vitro* hydrolysis experiments involving a microsomal fraction and a homogenate of human small intestinal mucosa, the Panel concluded that the flavouring substance can be anticipated to be readily hydrolysed upon consumption. Therefore, its safety assessment can be based on the individual amino acids in chemical group 34 and EFSA opinions FGE.26Rev1 and FGE.79.

6. Exposure Assessment (Appendix C)

All data necessary for the calculation of normal and maximum occurrence levels for refined sub categories of foods and beverages are reported in Appendix C.

6.1. Concentration in Processed and Non-processed Foods from Natural Occurrence

The candidate substance γ-glutamyl-valyl-glycine [FL-no: 17.038] is a synthetic substance. It has been identified as a natural component in vegetable and animal materials and has been quantitated by LC/MS/MS in several food sources such as beer (0.09 - 0.18 mg/kg), cheese (0.35 - 0.59 mg/kg), fish sauce 0.36 - 11.14 mg/kg), soy sauce (1.34 - 5.78 mg/kg) and scallops (0.08 - 0.77 mg/kg) (Flavour Industry, 2013). The exposure scenarios include the estimated intake from such natural sources, which, however, is low as compared to the normal and maximum intake as a flavour (Appendix C).

6.2. Non-food Sources of Exposure

The Panel has no information of any non-food sources of exposure to the candidate substances. However, it could conceivably be used to modify the bitterness of pharmaceuticals that contain bitter components. This is not anticipated at this stage (Flavour Industry, 2014).

6.3. Chronic Dietary Exposure

The exposure estimate to be used in the safety assessment of the candidate substance is the Added Portions Exposure Technique (APET) approach defined in the EFSA scientific opinion "Guidance on the data required for the risk assessment of flavourings to be used in or on foods"[6].

The chronic APET will be calculated both for adults and children and the highest value among these will be used as the exposure estimate to be compared to the Threshold of Toxicological Concern (TTC) value in the Procedure.

The highest chronic exposure is 231 µg/kg body weight (bw)/day derived from the scenario of a 15 kg three year old child consuming one portion of category 14.1 (Non-alcoholic ("soft") beverages) and one portion of category 12.5 (Soups and broths). This intake will result in a chronic APET[7] of 3465 µg/child per day (231 µg/kg bw per day for a 15 kg child) (for calculation of the chronic APET, see Appendix C).

[5] Commission Regulation (EC) No 1565/2000 of 18 July 2000 laying down the measures necessary for the adoption of an evaluation programme in application of Regulation (EC) No 2232/96 of the European Parliament and of the Council. OJ L 180, 19.7.2000, p. 8-16.

[6] EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids; Guidance on the data required for the risk assessment of flavourings. EFSA Journal 2010; 8(6):1623. [38pp.]. doi:10.2093/j.efsa.2010.1623.

[7] Excluding sub-categories 13.4, 14.2.1, 14.2.2, 14.2.3 and 14.2.4. Standard portion sizes for children are obtained by multiplying the adult standard portion sizes by a factor of 0.63.

Table 2: APET – Chronic Dietary Exposure

APET	Added[(a)] (µg/kg bw/day)		Other dietary sources[(b)] (µg/kg bw/day)		Combined[(c)] (µg/kg bw/day)	
Use level	**Normal**	**Maximum**	**Average**	**Maximum**	**Normal**	**Maximum**
Adults[(d)]	92	n.a.	0	n.a	92	n.a
Children[(e)]	231	n.a.	0	n.a.	231	n.a.
(Infants)[(f)]	The exposure to infants is currently under consideration in the EFSA exposure working group					

n.a. not applicable: the chronic APET calculation is based on the combined normal occurrence level.
(a) APET Added is calculated on the basis of the amount of flavour added to a specific food category.
(b) APET Other Dietary Sources is calculated based on the natural occurrence of the flavour in a specified food category.
(c) APET Combined is calculated based on the combined amount of added flavour and naturally occurring flavour in a specified food category.
(d) For the adult APET calculation a 60 kg person is considered representative.
(e) For the child APET calculation a 3-year old child with a 15 kg bw is considered representative
(f) For the infant (0 - 12 months) (and young children, 12 - 36 months) APET calculation a 12 months child with a 10 kg bw is considered representative.

Despite the fact that the substance is not intended to be used in food categories specifically intended for infants and young children (food category 13.2a - 13.2h), they will also consume food from the general food categories which may contain the substance. However, because the safety assessment of the substance is based on exposure to the separate amino acids, the Panel considers this to be of no safety concern.

6.4. Acute Dietary Exposure

The highest acute dietary exposure is 1260 µg/kg bw derived from the scenario of a 15 kg three year old child consuming three portions (3 x 200 x 0.63 = 378 g) of category 12.5 (soups and broths) which contain the maximum concentration of [FL-no: 17.038] (see Appendix C).

Table 3: APET – Acute Dietary Exposure

FL-no:	APET Added (µg/kg bw)		APET Other dietary sources (µg/kg bw)		APET Combined (µg/kg bw)	
Use level	**Normal**	**Maximum**	**Average**	**Maximum**	**Normal**	**Maximum**
Adults	n.a.	500	n.a.	0	n.a.	500
Children	n.a.	1260	n.a.	0	n.a.	1260
(Infants)	The exposure to infants is currently under consideration in the EFSA exposure working group					

6.5. Cumulative Dietary Exposure (Appendix B)

Structurally and metabolically related flavouring substances

γ-Glutamyl-valyl-glycine is a tripeptide which readily hydrolyses into three amino acids, *L*-glutamic acid, *L*-valine and glycine in the homogenate of human small intestinal mucosa and in the microsomal fraction from human small intestinal mucosa. *L*-glutamic acid, *L*-valine and glycine are all three macronutrients, which are normal components of protein.

7. Genotoxicity (Appendix D)

Structure alerts

No structure alerts have been identified by the methods of Ashby and Tennant (Ashby and Tenant, 1991) and Benigni et al. (Benigni et al., 2008).

7.1. Genotoxicity Studies

7.1.1. *In vitro*

Bacterial reverse mutation assay

In order to examine the mutagenic potential of γ-glutamyl-valyl-glycine [FL-no: 17.038], a reverse mutation assay was conducted in *Salmonella typhimurium* TA100, TA1535, TA98 and TA1537, and *Escherichia coli* WP2 uvrA with and without metabolic activation by the pre-incubation method. Water for injection was used as the vehicle for the test article (Oguma, 2010). The GLP study was conducted according to OECD Guideline 471.

A preliminary experiment was conducted to determine the concentration levels to be used in the main experiment. All strains were exposed to concentrations between 19.5 and 5000 µg/plate. Based on these results the minimum concentration which showed growth inhibition was selected as the maximum concentration for the main test. The main test was conducted with six concentrations between 39.1 and 1250 µg/plate for *S. typhimurium* TA1537 without metabolic activation, while in the presence of metabolic activation the concentrations were between 156 and 5000 µg/plate. For *S. typhimurium* TA98, TA100, TA1535, and *E. coli* WP2 uvrA with or without metabolic activation the concentrations tested were between 313 to 5000 µg/plate. The test was conducted twice using the same concentrations.

Precipitation on the plate and coloration by the test article in the test systems were not observed at any dose levels with or without metabolic activation.

In the observation of bacteria using a stereoscopic microscope, growth inhibition was observed at 1250 µg/plate and above for *S. typhimurium* TA1537 without metabolic activation, and at 5000 µg/plate for *S. typhimurium* TA1537 with metabolic activation.

In the main experiments it was not observed any increase nor a dose related response in the number of revertant colonies compared to negative controls in any test system with or without metabolic activation. The positive control compounds for each tester strain showed the expected mutagenic activity, indicating that the study was conducted properly.

It was concluded that γ-glutamyl-valyl-glycine [FL-no: 17.038] did not induce gene mutations in bacteria, under the conditions employed.

Chromosome aberration test

A chromosome aberration study was conducted using cultured Chinese hamster lung fibroblast (CHL/IU) cells to examine whether γ-glutamyl-valyl-glycine [FL-no: 17.038] has the potential to induce chromosome aberrations (Sono, 2010). The GLP study was conducted according to OECD Guideline 473.

Short-term treatment (5 + 18 hours, with and without S9-mix) and continuous treatment (24 and 48 hours, without S9-mix) methods were used. Based on the results of the cell-growth inhibition test, the study was conducted at three concentrations (775, 1550 and 3100 µg/mL). No cytotoxic effects were observed at the three concentrations used, while cell-growth inhibition exceeding 50 % was observed above 3100 µg/mL selected as the highest concentration. At all concentrations and conditions applied,

the frequencies of structural and numerical chromosomal aberration were comparable with the current negative control and were within historical control values.

Thus, γ-glutamyl-valyl-glycine [FL-no: 17.038] did not show any potential to induce structural or numerical (polyploidy) chromosome aberrations under the conditions of this study.

7.1.2. *In vivo*

Mouse micronucleus test

In order to examine whether γ-glutamyl-valyl-glycine [FL-no: 17.038] has clastogenic potential, a micronucleus study was conducted in Crlj:CD1(ICR)SPF mice (Ishii, 2010). The GLP study was conducted according to OECD Guideline 474.

[FL-no: 17.038] was administered to the animals twice orally at an approximately 24-hour interval at 500, 1000 or 2000 mg/kg bw/day and bone marrows smear specimens were prepared at approximately 24 hours after the second administration. A negative control group received water for injection twice and a positive control was treated with mitomycin C once at 1 mg/kg.

In the test article administration groups, the proportion of MNPCE was 0.15 ± 0.06 % in the 500 mg/kg/day group, 0.15 ± 0.04 % in the 1000 mg/kg/day group and 0.08 ± 0.03 % in the 2000 mg/kg/day. In comparison between these values and the value of 0.09 ± 0.07 % in the negative control group, there was no statistically significant increase in any test article administration group and there were no dose-related changes. The proportion of PCE in 200 entire erythrocytes in each test article administration group did not show any statistically significant change when compared to the negative control group. In this study, exposure of bone marrow to the test article was not demonstrated; however, since examination was conducted up to the highest concentration of 2000 mg/kg/day, which was described in the toxicity study guidelines, and since there is no indication for genotoxicity from the *in vitro* data a substantiation of target tissue exposure is not required. The proportion of MNPCE in the negative and positive control groups were within the range of each background data of the test facility.

In conclusion, γ-glutamyl-valyl-glycine [FL-no: 17.038] was judged to have no potential to induce structural or numerical (aneuploidy) chromosome aberrations in bone marrow of mice under the conditions of this study.

7.2. Conclusion on genotoxicity

There was no indication of genotoxicity in the bacterial reversion assays, in an *in vitro* assay or in an *in vivo* micronucleus test. Thus, there is no safety concern with respect to genotoxicity for the candidate substance.

8. Absorption, Distribution, Metabolism and Elimination

No data are available on absorption or distribution of the tripeptide. It is presumably absorbed by carrier-mediated metabolism, but no data are provided.

The hydrolysis of the candidate substance γ-glutamyl-valyl-glycine [FL-no: 17.038] by various hydrolytic enzymes in (simulated) stomach and gut preparations was investigated *in vitro* in order to find out whether assessment of the candidate substance could be based on its hydrolysis products, the amino acids glycine, *L*-valine and *L*-glutamate; enzymes expected to be involved are (γ-glutamyl)-peptidases (Flavour Industry, 2013).

Hydrolysis in a *simulated gastric fluid* at pH 1.18 indicated that [FL-no: 17.038] is not hydrolysed by added porcine pepsin (Sigma P6887; 6.4 mg/ml: "2-fold concentrated") during 6 hours at 37 °C. The

concentration of [FL-no: 17.038] was based on the expected concentration after oral consumption of [FL-no: 17.038]-containing food, 19 ppm or 62 µM (Flavour Industry, 2013).

Hydrolysis in *simulated intestinal fluid* at pH 7.5 indicated that [FL-no: 17.038] was hydrolysed by added porcine pancreatine (Sigma P8096; 20 mg/ml: "2-fold concentrated"); after 6 hours, some 60 % had been hydrolysed at 37 °C. The dipeptide γ-Glu-Val remained below detection but some 20 % was present as the Val-Gly dipeptide after 6 hours. The concentration of [FL-no: 17.038] was based on the presumed intestinal concentration after oral consumption of [FL-no: 17.038]-containing food, 15 µM (Flavour Industry, 2013).

Hydrolysis by a *microsomal fraction from human small intestinal mucosa* (XenoTech Co.Ltd; a pooled preparation from male and female human mucosa) at pH 8.0 indicated that [FL-no: 17.038] was rapidly hydrolysed by the microsomal preparation (8 mg/ml microsomal protein); already after 10 minutes, 97 % had been hydrolysed, while 74 % was identified as the Val-Gly dipeptide. After 15 minutes, the tripeptide was below the level of detection, while the Val-Gly dipeptide started to decrease. After 60 minutes, 39 % was still present as the dipeptide Val-Gly; the remainder presumably is in the form of the individual amino acids. γ-Glu-Val could not be detected during the incubation. The concentration of [FL-no: 17.038] in the incubation was based on the presumed intestinal concentration after oral consumption of [FL-no: 17.038]-containing food, 15 µM (Flavour Industry, 2013).

Hydrolysis by a *homogenate of human small intestinal mucosa* (XenoTech Co Ltd; no details available) at pH 8.0 indicated that [FL-no: 17.038] was rapidly hydrolysed by the homogenate of human intestinal mucosa (3.2 mg/ml protein); after 10 minutes, 42 % was hydrolysed, while 21 % was present as Val-Gly. After 60 minutes, 97 % was hydrolysed, while 7 % was still present as Val-Gly. Very low levels of the γ-Glu-Val dipeptide (less than 1 %) could be detected during the incubation. The initial concentration of [FL-no: 17.038] in the incubation was 15 µM (Flavour Industry, 2013).

In order to assess the capacity of the intestine to hydrolyse the candidate substance [FL-no: 17.038] after oral administration in humans to its individual amino acids, the data of Bruyère et al (2010) were used These authors estimated that the microsomal protein content of scraped off human intestinal mucosa remained constant along the length of the small intestine, at about 1.55 mg/cm intestine (Bruyère et al., 2010). Using the data on hydrolysis of the candidate substance [FL-no: 17.038] by the microsomal fraction from human small intestinal mucosa (see above), it can be calculated[8] that at pH 8.0 the peptide would be very rapidly hydrolysed *in vivo* after oral administration. It is not clear whether the hydrolysing enzymes *in vivo*, as recovered in the microsomal fraction, are present in the membranes at the *luminal surface* of the mucosal cells (which will be recovered in the microsomal fraction) or *inside* the mucosal cell; in the latter case the peptide would have to be taken up by the cell by carriers before it could be hydrolysed.

In summary, it can be assumed that the candidate substance γ-glutamyl-valyl-glycine [FL-no: 17.038] is rapidly hydrolysed to its individual amino acids after oral administration with the food.

9. Toxicity Data (Appendix F)

9.1. 28-Day Dietary Administration Systemic Toxicity Study in Rats

In order to examine the toxicity of the candidate substance γ-glutamyl-valyl-glycine [FL-no: 17.038], a 28 days toxicity study according to OECD Guideline 407 was conducted (a GLP study), including full histopathology of the high dose group (Okamura, 2010). [FL-no: 17.038] was mixed with basal powder diet and given to Sprague-Dawley strain SPF rats [Crl:CD(SD), 10 males and 10 females per dose, six weeks of age at the start of administration] by 28-day dietary administration at dose levels of 100, 300 and 1000 mg/kg bw/day. A control group received the basal powder diet alone in the same

[8] See Appendix E for the calculation.

manner. The rats were housed individually. The mean intake of [FL-no: 17.038] in each test article administration group during the administration period was 114, 336 and 1113 mg/kg bw/day in males and 114, 328 and 1124 mg/kg bw/day in females in the 100, 300 and 1000 mg/kg bw/day groups, respectively.

No deaths occurred during the administration period, and there were no changes that were thought to be attributable to administration of [FL-no: 17.038] observed in clinical signs, detailed clinical observations, manipulative test, grip strength, motor activity, body weights, food consumption, water intake, ophthalmology, hematology, necropsy or histopathology in any tissue examined.

In urinalysis, a tendency toward increase in the number of animals with positive protein was observed in males in the 300 and 1000 mg/kg groups, but not in females. However, it was judged to have no toxicological significance since there were no changes in the blood chemistry examination suggesting damage in kidney function and there were no changes in the (histo-)pathological examinations. None of the other 13 urinalysis parameters was changed.

In blood chemistry examination, no changes were observed except for slight but statistically significant decreases (16 %) in urea nitrogen and creatinine in males in the 1000 mg/kg group, which were judged to have no toxicological significance since they were *not increased* but rather decreased compared to controls.

There were no changes in organ weights except for statistically significant lower values (17 - 20 %) in the relative and absolute weights of the spleen in females in the 100 and 1000 mg/kg groups. However, the changes were within the range of the historical controls of Bozo Research Center Inc.. They were judged to have no toxicological significance since hematological and histopathological examination of the lymphatic/hematopoietic organs including the spleen showed no changes.

The No Observed Adverse Effect Level (NOAEL) of [FL-no: 17.038] under the conditions of this 28-day study was higher than 1000 mg/kg bw/day in both sexes.

The Panel considered that sub-chronic toxicity studies are not needed for the evaluation of γ-glutamyl-valyl-glycine [FL-no: 17.038] in the present case as the evaluation is solely based on the ready hydrolysis of the tripeptide into the respective amino acids upon digestion.

10. Exposure Compared to TTC

Not applicable.

11. Procedure for Assessment / Safety Assessment

As the consumption of the single amino acids from which the candidate substance is build is orders of magnitude higher than the intake arising from the use as flavouring substance, the Panel decided not to carry out the safety assessment according to the Procedure (Appendix A). Instead the below listed statements lead to the final conclusion for the candidate substance.

- The candidate substance [FL-no: 17.038] presents no safety concern with respect to genotoxicity.
- It can be assumed that the candidate substance [FL-no: 17.038] is rapidly hydrolysed to its individual amino acids, *L*-glutamic acid, *L*-valine and glycine, after oral intake with the food.
- Of these endogenous amino acids, two (glycine and *L*-valine) are evaluated in existing FGE's (FGE.26Rev1 (EFSA, 2008a) and FGE.79 (EFSA, 2008b)). *L*-glutamic acid was evaluated by the JECFA (JECFA no 1420) (JECFA, 2006).
- To assess the (margin of) safety for the intake of the amino acids released from [FL-no: 17.038] the following can be considered. The generally accepted human daily allowance of protein is 700

mg/kg bw/day ((Institute of Medicine, 2005): dietary reference intake tables), i.e. 42.000 mg protein (60 kg person) in the form of amino acids. The APET estimate for [FL-no: 17.038] adds 5.5 mg/day in the form of amino acids Gly, Val and Glu. Of this total protein intake, approx. 6.5%, i.e. 2730 mg, is the essential amino acid *L*-Valine (www.proteinsandproteomics.org/content/free/tables_1/table08.pdf); the intake of valine through [FL-no: 17.038] adds approximately 2 mg to this amount. The (minimum) requirement for *L*-valine is 10 mg/kg bw/day (WHO, 1985). Thus, the human exposure of these three endogenous amino acids through food is orders of magnitude higher than the anticipated levels of exposure from their use as flavouring substances derived from γ-glutamyl-valyl-glycine [FL-no: 17.038].

Therefore, these three substances were not taken through the Procedure and the Panel concluded that these substances were not of safety concern at their estimated levels of intake as flavouring substances. The Panel therefore also conclude for the candidate substance γ-glutamyl-valyl-glycine [FL-no: 17.038] that it is not of safety concern at its estimated level of intake as flavouring substance.

12. Margin of Safety

Not applicable.

CONCLUSION

The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids of the European Food Safety Authority was requested to evaluate one flavouring substance (candidate substance) in the Flavouring Group Evaluation 401, using the Procedure in Commission Regulation (EC) No 1331/2008.

The candidate substance γ-glutamyl-valyl-glycine [FL-no: 17.038] is a synthetic substance. It has also been identified as a natural component in vegetable and animal materials and has been quantitated in some unprocessed and processed food.

According to the applicant the candidate substance γ-glutamyl-valyl-glycine [FL-no: 17.038] is intended to be used to modify and balance the total flavour bringing out the middle to afternote and adds brothy, creamy flavours similar to that of well-cooked stew.

The dietary exposure to the candidate substance is calculated to be 92 µg/kg bw/day in a 60 kg adult and 231 µg/kg bw/day in a 15 kg three-year old child. In per capita terms, these are 5500 µg/capita/day and 3465 µg/capita/day, respectively. Despite the fact that the substance is not intended to be used in food categories specifically intended for infants and young children, they will also consume food from the general food categories which may contain the substance. However, because the safety assessment of the substance is based on exposure to the separate amino acids, the Panel considers this to be of no safety concern.

The highest acute exposure value of 1.26 mg/kg bw derived from the scenario of a 15 kg three year old child consuming three portions (3 x 200 x 0.63 = 378 g) of category 12.5 (soups and broths) all of which contain the maximum concentration of [FL-no: 17.038]. This is considered not to be of safety concern.

There was no indication of genotoxicity in the bacterial reversion assays, in an *in vitro* chromosome aberration assay and in an *in vivo* micronucleus test. Based on these data there is no safety concern with respect to genotoxicity for the candidate substance. In a 28-day oral study in rats the no observed adverse effect level (NOAEL) of [FL-no: 17.038] was 1000 mg/kg bw/day (the highest dose) in both sexes. No 90 days study will be required as the evaluation is solely based on the hydrolysis of the tripeptide into amino acids.

Since it can be assumed that the candidate substance [FL-no: 17.038] is rapidly hydrolysed to its individual amino acids after oral administration with the food, the Panel based its risk assessment on

the hydrolysis products, the three individual amino acids, *L*-glutamic acid (evaluated by the JECFA, JECFA no 1420), *L*-valine [FL-no: 17.028] (evaluated by the EFSA in FGE.26Rev1) and glycine ([FL-no: 17.034] (evaluated by the JECFA, JECFA no 1421 and considered by the EFSA in FGE.79). The human exposure to these three endogenous amino acids, through food is orders of magnitude higher than the anticipated levels of exposure from their use as flavouring substances. Therefore, these three substances were not taken through the Procedure. The Panel concluded that the substances were not of safety concern at their estimated levels of intake as flavouring substances.

The specifications for [FL-no: 17.038] are considered adequate according to Commission Regulation (EC) no 1334/2008.

Based on the above considerations, the candidate substance, γ-glutamyl-valyl-glycine [FL-no: 17.038] is also not considered to be of safety concern at its estimated level of intake as flavouring substance.

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DOCUMENTATION PROVIDED TO EFSA

1. Complete technical dossier for a new flavouring substance. Submission to the European Food Safety Authority (EFSA) in accordance with Regulations (EC) No.1331/2008, (EC) No. 1334/2008 and No. 2232/96 and with the Guidance provided by EFSA. Received June 2013.

2. Response to EFSA question no. EFSA-Q-2013-00409 regarding the pH values and purities used. Received 25 February 2014.

HISTORY OF EVALUATION

Not applicable.

APPENDIX

Appendix A. Procedure Scheme

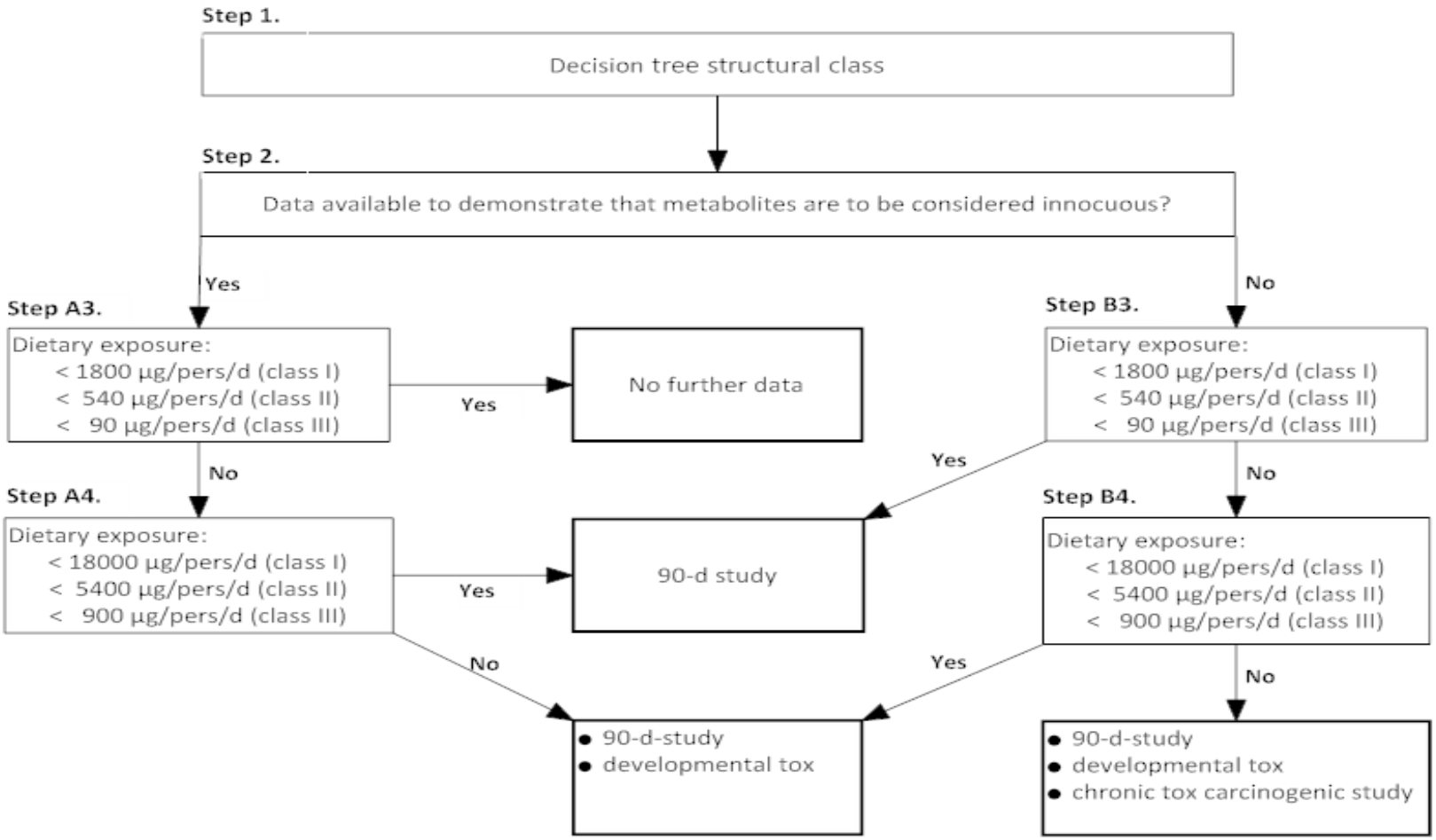


Figure A.2 *Procedure for Safety Evaluation of Individual Flavouring Substances*

Appendix B. Structurally and Metabolically Related Substances

Hydrolysis products of the candidate substance [FL-no: 17.038] were identified among the flavouring substances that have already been evaluated by EFSA or JECFA. These are the amino acids evaluated: L-glutamic acid (evaluated by JECFA, JECFA no 1420), L-valine [FL-no: 17.028] (evaluated in FGE.26Rev1 (EFSA, 2008a)) and glycine ([FL-no: 17.034] evaluated by JECFA, JECFA no 1421 (JECFA, 2006) and considered by EFSA in FGE.79 (EFSA; 2008b)).

Appendix C. Use Levels and Exposure Calculations

Table 4: Normal and Maximum Occurrence Levels for Sub-Categories of Foods and Beverages

CODE X code	Food categories §	Standard portions* (g)	Occurrence level as added flavouring (mg/kg)		Occurrence level from other sources @ (mg/kg)		Combined occurrence level from all sources # (mg/kg)	
			Normal	Maximum	Normal[$]	Maximum	Normal	Maximum
01.1	Milk and dairy-based drinks	200	15	45			15	45
01.2	Fermented and renneted milk products (plain), excluding food category 01.1.2 (dairy-based drinks)	200	15	45			15	45
01.3	Condensed milk and analogues (plain)	70	20	50			20	50
01.4	Cream (plain) and the like	15	20	50			20	50
01.5	Milk powder and cream powder and powder analogues (plain)	30	15	45			15	45
01.6	Cheese and analogues	40			0.47	0.59	0.47	0.59
01.7	Dairy-based desserts (e.g., pudding, fruit or flavoured yoghurt)	125	15	45			15	45
01.8	Whey and whey products, excluding whey cheeses	200						
02.1	Fats and oils essentially free from water	15	30	60			30	60
02.2	Fat emulsions mainly of type water-in-oil	15	30	60			30	60
02.3	Fat emulsions mainly of type water-in-oil, including mixed and/or flavoured products based on fat emulsions	15	30	60			30	60
02.4	Fat-based desserts excluding dairy-based dessert products of category 1.7	50	15	45			15	45
03.0	Edible ices, including sherbet and sorbet	50						
04.1.1	Fresh fruit	140						
04.1.2	Processed fruit	125						
04.1.2.5	Jams, jellies, marmalades	30						
04.2.1	Fresh vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), seaweed, and nut and seed	200						
04.2.2	Processed vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), seaweed, and nut and seed purees and spreads (e.g. peanut butter) and nuts and seeds	200						
04.2.2.5	Vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), seaweed, and nut and seed purees and spreads (e.g. peanut butter)	30						

CODE X code	Food categories §	Standard portions* (g)	Occurrence level as added flavouring (mg/kg)		Occurrence level from other sources @ (mg/kg)		Combined occurrence level from all sources # (mg/kg)	
			Normal	Maximum	Normal[$]	Maximum	Normal	Maximum
05.1	Cocoa products and chocolate products, including imitations and chocolate substitutes	40	30	60			30	60
05.1.3	Cocoa-based spreads, including fillings	30						
05.2	Confectionery, including hard and soft candy, nougats, etc., other than 05.1, 05.3 and 05.4	30						
05.3	Chewing gum	3						
05.4	Decorations (e.g. for fine bakery wares), toppings (non-fruit) and sweet sauces	35						
06.1	Whole, broken or flaked grain, including rice	200						
06.2	Flours and starches (including soya bean powder)	30						
06.3	Breakfast cereals, including rolled oats	30	80	160			80	160
06.4	Pastas and noodles and like products (e.g. rice paper, rice vermicelli, soya bean pastas and noodles)	200						
06.5	Cereal and starch based desserts (e.g. rice pudding, tapioca pudding)	200						
06.6	Batters (e.g. for breading or batters for fish or poultry)	30						
06.7	Pre-cooked or processed rice products, including rice cakes (Oriental type only)	200						
06.8	Soya bean products (excluding soya bean products of food category 12.9 and fermented soya bean products of food category 12.10)	100						
07.1	Bread and ordinary bakery wares	50						
07.2	Fine bakery wares (sweet, salty, savoury) and mixes	80	30	60			30	60
08.1	Fresh meat, poultry and game	200						
08.2	Processed meat, poultry and game products in whole pieces or cuts	100	15	45	0.85	0.85	15.85	45.85
08.3	Processed comminute meat, poultry and game products	100	15	45			15	45
08.4	Edible casings (e.g. sausage casings)	1	15	45			15	45
09.1.1	Fresh fish	200						
09.1.2	Fresh molluscs, crustaceans and echinoderms	200			0.5	0.77	0.5	0.77
09.2	Processed fish and fish products, including molluscs, crustaceans and echinoderms	100						
09.3	Semi-preserved fish and fish products, including molluscs,	100						

CODE X code	Food categories §	Standard portions* (g)	Occurrence level as added flavouring (mg/kg)		Occurrence level from other sources @ (mg/kg)		Combined occurrence level from all sources # (mg/kg)	
			Normal	Maximum	Normal[$]	Maximum	Normal	Maximum
	crustaceans and echinoderms							
09.4	Fully preserved, including canned or fermented, fish and fish products, including molluscs, crustaceans and echinoderms	100			0.1	0.17	0.1	0.17
10.1	Fresh eggs	100						
10.2	Egg products	100						
10.3	Preserved eggs, including alkaline. salted and canned eggs	100						
10.4	Egg-based desserts (e.g. custard)	125						
11.1	Refined and raw sugar	10						
11.2	Brown sugar excluding products of food category 11.1	10						
11.3	Sugar solutions and syrups, and (partially) inverted sugars, including molasses and treacle, excluding products of food category 11.1.3 (soft white sugar, soft brown sugar, glucose syrup, dried glucose syrup, raw cane sugar)	30						
11.4	Other sugars and syrups (e.g. xylose, maple syrup, sugar toppings)	30						
11.5	Honey	15						
11.6	Table-top sweeteners, including those containing high-intensity sweeteners	1						
12.1	Salt and salt substitutes	1						
12.2	Herbs, spices, seasonings and condiments (e.g. seasoning for instant noodles)	1	80	160	4.45	11.14	84.45	171.14
12.3	Vinegars	15						
12.4	Mustards	15						
12.5	Soups and broths	200	20	50			20	50
12.6	Sauces and like products	30	20	50			20	50
12.7.a	Salads 120 g (e.g. macaroni salad, potato salad) excluding cocoa- and nut-based spreads of food categories	120						
12.7.b	Sandwich spreads (20 g), excluding cocoa- and nut-based spreads of food categories	20						
12.8	Yeast and like products	1	15	45			15	45
12.9	Soybean-based seasonings and condiments	15						

CODE X code	Food categories §	Standard portions* (g)	Occurrence level as added flavouring (mg/kg)		Occurrence level from other sources @ (mg/kg)		Combined occurrence level from all sources # (mg/kg)	
			Normal	Maximum	Normal[$]	Maximum	Normal	Maximum
12.9.2	Soybean sauce	15						
12.9.3	Fermented soybean sauce	15						
12.9.1	Fermented soya bean products (e.g. miso)	40						
12.10	Protein products other than from soybeans	15			3.93	5.78	3.93	5.78
13.2. a	Complementary foods for infants and young children: Dry instant cereals (with or without milk), including pasta	110						
13.2. b	Complementary foods for infants and young children: Meat based or fish based dinner	170						
13.2. c	Complementary foods for infants and young children: Dairy based dessert	110						
13.2. d	Complementary foods for infants and young children: Vegetables, potatoes, broth, soups, pulses	170						
13.2. e	Complementary foods for infants and young children: Biscuits and cookies	20						
13.2. f	Complementary foods for infants and young children: Fruit purée	110						
13.2. g	Complementary foods for infants and young children: Fruit juice	120						
13.2. h	Milk for young children	200						
13.3	Dietetic foods intended for special medical purposes (excluding food products of category 13.1 “Infant formulae, follow-up formulae and other formulae for special medical purposes for infants”)	200						
13.4¤	Dietetic formulae for slimming purposes and weight reduction	200						
13.5	Dietetic foods (e.g. supplementary foods for dietary use), excluding products of food categories 13.1 (Infant formulae, follow-up formulae and other formulae for special medical purposes for infants”), 13.2–13.4 and 13.6	200						
13.6	Food supplements	5						
14.1a	Coffee powder	12						
14.1b	Drinks mix powders	30						
14.1c	Other non-alcoholic (“soft”) beverages (expressed as liquid)	300	5	15			5	15
14.2.1¤	Beer and malt beverages	300			0.13	0.18	0.13	0.18
14.2.2¤	Cider and perry	300						

CODE X code	Food categories §	Standard portions* (g)	Occurrence level as added flavouring (mg/kg)		Occurrence level from other sources @ (mg/kg)		Combined occurrence level from all sources # (mg/kg)	
			Normal	Maximum	Normal$	Maximum	Normal	Maximum
14.2.3¤	Grape wines	150						
14.2.4¤	Wines (other than grape)	150						
14.2.5¤	Mead	150						
14.2.6¤	Distilled spirituous beverages containing more than 15 % alcohol	30						
14.2.7¤	Aromatized alcoholic beverages (e.g., beer, wine and spirituous cooler-type beverages, low alcoholic refreshers)	300						
15.1	Snacks, potato-, cereal-, flour- or starch-based (from roots and tubers, pulses and legumes)	30	80	160			80	160
15.2	Processed nuts, including coated nuts and nut mixtures (with e.g. dried fruit)	30						
15.3	Snacks – fish based	30						
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) – foods that could not be placed in categories 01–15	300						

§ Most of the categories reported are the sub-categories of Codex GSFA (General Standard for Food Additives, available at http://www.codexalimentarius.net/gsfaonline/CXS_192e.pdf) used by the JECFA in the SPET technique (FAO/WHO, 2008). In the case of category 13.2 (complementary foods for infants and young children), further refined categories have been created so that a specific assessment of dietary exposure can be performed in young children.

* In case of foods marketed as powder or as concentrates, occurrence levels must be reported for the reconstituted product, considering the instructions reported on the product label or one of the standard dilution factors established by the JECFA (FAO/WHO 2008):

- 1/25 for powder used to prepare water-based drinks such as coffee, containing no additional ingredients,
- 1/10 for powder used to prepare water-based drinks containing additional ingredients such as sugars (ice tea, squashes, etc.),
- 1/7 for powder used to prepare milk, soups and puddings,
- 1/3 for condensed milk.

$ In order to estimate normal values in each category, only foods and beverages in which the substance is present in significant amount will be considered (e.g. for the category "Fresh fruit" 04.1.1., the normal concentration will be the median concentration observed in all kinds of fruit where the flavouring substance is known to occur).

The normal and maximum combined occurrence levels of the substance will be assessed by the applicant either by adding up occurrence levels from added use to that from other sources or by expert judgment based on the likelihood of their concomitant presence. This will be done both for normal use levels and for maximum use levels.

@ As natural constituent and/or developed during the processing and/or as carry over resulting from their use in animal feed.

¤The sub-categories 14.2.1, 14.2.2, 14.2.3, 14.2.4, 14.2.5, 14.2.6 and 14.2.7 ("Alcoholic beverages") and the sub-category 13.4 ("Dietetic formulae for slimming purposes and weight reduction") are a priori not consumed by children.

DIETARY EXPOSURE TO [FL-NO: 17.038] FROM THE CONSUMPTION OF FLAVOURED FOODS AND BEVERAGES IN ADULTS AND CHILDREN

Chronic Dietary Exposure

ADULTS ("Added Portions Exposure Technique" [APET][9]).

On the Basis of Normal Occurrence Level from Added Flavourings

Food sub-categories resulting in the highest potential dietary exposure:

Beverage: The maximum intake will be from category 14.1 (Non-alcoholic ("soft") beverages). The normal combined occurrence level of 5 mg/kg in of 300 g of non-alcoholic beverages gives an intake of 1500 µg/person per day.

Solid Food: The maximum intake will be from category 12.5 (Soups and broths). The normal combined occurrence level of 20 mg/kg in 200 g of soups and broths gives an intake of 4000 µg/person per day.

Chronic APET: 5500 µg/person per day (92 µg/kg bw per day for a 60 kg person).

CHILDREN (3-year old child of 15 kg body weight)

Food sub-categories resulting in the highest potential dietary exposure:

Beverage: The maximum intake will be from category 14.1 (Non-alcoholic ("soft") beverages). The normal combined occurrence level of 5 mg/kg in of 300 g of non-alcoholic beverages gives an intake of 945 µg/child per day (5 mg/kg x 300 g x 0.63).

Solid Food: The maximum intake will be from category 12.5 (Soups and broths). The normal combined occurrence of 20 mg/kg in 200 g of soups and broths gives an intake of 2520 µg/child per day (20 mg/kg x 200 g x 0.63).

Chronic APET[10]: 3465 µg/child per day (231 µg/kg bw per day for a 15 kg child).

Conclusion

The chronic APET values are 92 µg/kg bw per day for 60 kg adults and 231 µg/kg bw per day for 15 kg child. In terms of per kg bw, the children value of 3465 µg/day is the higher.

[9] The APET has been calculated based on the occurrence levels in the food sub-categories reported in the above Table**,** with the exclusion of categories 13.2 (complementary foods for infants and young children).

[10] Excluding sub-categories 13.4, 14.2.1, 14.2.2, 14.2.3, 14.2.4, 14.2.5, 14.2.6 and 14.2.7. Standard portion sizes for children are obtained by multiplying the adult standard portion sizes by a factor of 0.63.

INFANTS AND YOUNG CHILDREN

According to the Applicant [FL-no: 17.038] will not be used in any of the the food categories 13.2a – 13.2h (complementary foods for infants and young children).

Conclusion

No exposure to [FL-no: 17.038] is expected in any food item for infants and young children (< 3years of age).

Acute Dietary Exposure

ADULTS

The highest acute intake is assumed to result from the consumption of three portions[11] of category 12.5 (soups and broths) containing a maximum concentration of 50 mg/kg of [FL-no: 17.038]. This gives a value of 3 x 200 g x 50 mg/kg = 30 mg/person = 500 µg/kg for a 60 kg person.

CHILDREN[12]

The highest acute intake is assumed to result from the consumption of three portions (3 x 200 g x 0.63 = 378 g) of category 12.5 (soups and broths) containing a maximum concentration of 50 mg/kg of [FL-no: 17.038]. This gives a value of 378 g x 50 mg/kg = 18.9 mg/child = 1260 µg/kg for a 15 kg child.

INFANTS AND YOUNG CHILDREN (< 3 YRS): No intentional consumption is intended.

Conclusion

The highest[13] acute APET value is 1260 µg/kg bw derived from the scenario of a 15 kg three year old child consuming three portions (3 x 200 g x 0.63 = 378 g) of food category 12.5 (soups and broths) all of which contain the maximum concentration of [FL-no: 17.038].

Cumulative Dietary Exposure to [FL-no: 17.038]

The candidate substance γ-glutamyl-valyl-glycine [FL-no: 17.038] is considered to be rapidly hydrolysed before absorption to the three endogenous amino acids, L-glutamic acid (evaluated by JECFA (JECFA, 2006), JECFA no 1420), L-valine [FL-no: 17.028] (evaluated by EFSA (EFSA, 2008a) and glycine ([FL-no: 17.034] evaluated by JECFA, JECFA no 1421 (JECFA, 2006) and considered by EFSA in FGE.79 (EFSA, 2008b)). The exposure of these amino

[11] EFSA Journal 2010; 8(6):1623, Guidance on data submission for flavourings evaluation: In both adults and 3 year-old children, the acute exposure is represented by the consumption of three portions of either a solid food or a beverage, containing the flavouring substance at its maximum occurrence levels.
[12] Based on the same considerations as for adults but using the special factors used for chronic exposure to infants.
[13] The highest value obtained among adults and children of all ages.

acids through food is orders of magnitude higher than the anticipated level of exposure from the use of the candidate substance γ-glutamyl-valyl-glycine [FL-no: 17.038] as a flavouring substance.

It is therefore not considered applicable to calculate the cumulative dietary exposure.

Appendix D. Genotoxicity

Table 5: Summary of *in vitro* genotoxicity studies

FL-no JECFA-no	Union list name / test material	Test System	Test Object	Concentration	Result	Reference	Comments
17.038 2123	γ-Glutamyl-valyl-glycine	Ames test	*S.typhimuriun* TA98, TA100, TA1535, TA1537 *E.coli* WP2 *uvrA*	Up to 5 mg/plate (with and without metabolic activation S9-mix)	Negative	Oguma, 2010	Test in according with OECD Guideline 471 and GLP.
		Chromosome aberration test	Chinese hamster lung fibroblast (CHL/IU) cells	Up to 3100 µg/mL	Negative	Sono, 2010	Test in accordance with OECD Guideline 473 test and GLP.

Table 6: Summary of *in vivo* genotoxicity studies

FL-no JECFA-no	Union list name / test material	Test System	Test Object	Route	Dose	Result	Reference	Comments
17.038 2123	γ-Glutamyl-valyl-glycine	Micronucleus test	Mice	Oral	Up to 2000 mg/kg bw	Negative	Ishii, 2010	Test in accordance with OECD Guideline 474 and GLP.

Appendix E. Metabolism

According to the applicant, the anticipated usual use level of γ-glutamyl-valyl-glycine [FL-no: 17.038] in food is approximately 20 ppm or 66 μM. The applicant assumes a concentration of 5 mg/kg or 16 μM in the intestinal fluid released from the stomach.

In the incubation with a human intestinal microsomal preparation, after 10 minutes (at pH 8.0), 97 % of the tripeptide had been hydrolysed to the dipeptide Val-Gly; after 60 minutes, 60 % had been completely hydrolysed (Flavour Industry, 2013).

The concentration of (human) intestinal microsomal protein in the incubation was 8 mg/ml; the microsomal protein content of human intestine is 1.55 per cm (Bruyère et al., 2010). Thus, 8 mg of microsomal protein corresponds to 8/1.55, i.e. approximately five cm (of the total of seven meters) of human small intestine.

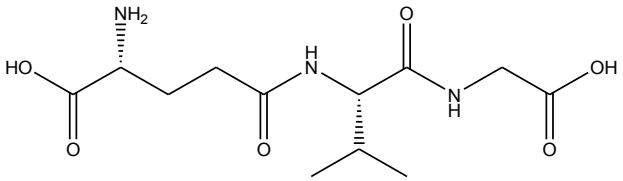
During 10 minutes presence of the intestinal fluid in five cm of small intestine over 90 % would be expected to be hydrolysed to the dipeptide. Therefore, the tripeptide will disappear very rapidly (within a few minutes), once in the small intestine. Complete hydrolysis would take longer: in the same five cm during 60 minutes, 60 % would be completely hydrolysed. However, as the intestinal fluid passes through the small intestine more microsomal protein would become recruited, so that over a length of 50 cm a 60 % complete hydrolysis of the dipeptide would only take six minutes. As the intestinal fluid moves through the small intestine, the hydrolysis would increase progressively till virtual completeness.

Table 7: Summary of the Procedure for Evaluation of Individual Flavouring Substances

FL-no	EU Register name	Structural formula	JECFA no CAS no	Procedure pathway (A or B)[(a)]	Structural class	Chronic APET µg/person/day (Adult or Child)[(b)]	Procedure step	Toxicological data required	EFSA comments
[17.038]	γ-glutamyl-valyl-glycine	*	- 38837-70-6	**	I	3465	-	-	-

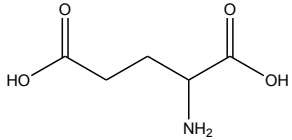
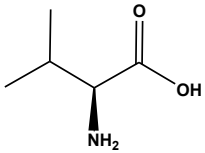
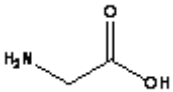
(a): Data available to demonstrate that metabolites are to be considered innocuous? Yes: A; No: B
(b): The highest chronic APET value among adults and children expressed in µg/person/day

*



** The human exposure of the three endogenous amino acids, L-glutamic acid (evaluated by JECFA, JECFA no 1420), L-valine [FL-no: 17.028] and glycine through food is orders of magnitude higher than the anticipated levels of exposure from their use as flavouring substances. Therefore, these three substances are not taken through the Procedure. However, the Panel concluded that the substances were not of safety concern at their estimated levels of intake as flavouring substances.

Table 8: Summary of Evaluation of Metabolism Products from the Flavouring Substance

FL-no JECFA-no	Name	Structural formula	Estimated amount MSDI (EU) µg/*capita*/day	EFSA status	Cramer class	EFSA Comments
- 1420	L-glutamic acid		267*	No evaluation as flavouring substance	III	L-glutamic acid is not used as a flavour in the EU. JECFA has evaluated the substance: No safety concern at the estimated level of intake as a flavouring substance.
17.028	L-Valine		18	No safety concern at the estimated level of intake as a flavouring substance.	I	
17.034 1421	Glycine		135	No safety concern at the estimated level of intake as a flavouring substance.	III	

*The 267 µg/*capita*/day originates from the JECFA figure of 313 µg/*capita*/day. JECFA assume that EU population is 320 x 10^6, EFSA use the figure 375 x 10^6. Therefore is the estimated amount (MSDI) in EU calculated to be: 313 µg/*capita*/day x (320 x 10^6 / 375 x 10^6) = 267 µg/*capita*/day.

Appendix F. Toxicity

Acute Oral Toxicity Study in Rats

None were submitted.

28-Day Range-Finding Systemic Dietary Toxicity Study in Rats

See section 9.1 and table 9.

90-Day Dietary Administration Systemic Toxicity Study in Rats

None were submitted.

Table 9: Summary of Toxicity studies

FL-no JECFA-no	Union list name / test material	Species; Sex No/group	Route of administration	Dose level Mg/kg bw/day	Duration	LD50 / NOAEL / BMDL (mg/kg bw/day)	Reference	Comments
17.038 2123	γ-Glutamyl-valyl-glycine	Sprague-Dawley rats: 10 M/10 F/dose level	Dietary	Calculated mean intake in each group were M: 114, 336 and 1113 mg/kg bw F: 114, 328 and 1124 mg/kg bw	28-days	NOAEL: > 1000 mg/ kg bw/ day	Okamura T, 2010	Range finding study. The administrated dose levels were 100, 300 and 1000 mg/ kg bw/day.

ABBREVIATIONS

APET	Added Portions Exposure Technique
BW	Body Weight
CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids Chemical Abstract Service
CoE	Council of Europe
EC	European Commission
EFSA	The European Food Safety Authority
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FEMA	Flavor and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
FLAVIS (FL)	Flavour Information System (database)
GC	Gas Chromatography
GLP	Good Laboratory Practice
GMO	Genetically Modified Organisms
HPLC	High Performance Liquid Chromatography
ID	Identity
IOFI	International Organization of the Flavour Industry
IR	Infrared spectroscopy
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LC	Liquid Chromatography
MS	Mass spectrometry
MSDI	Maximised Survey-derived Daily Intake
NMR	Nuclear Magnetic Resonance
No	Number
NOAEL	No Observed Adverse Effect Level
OECD	Organisation for Economic Co-operation and Development
RH	Relative Humidity
S9-MIX	A metabolic activation system with rat-liver microsome fraction plus cofactors
SCF	Scientific Committee on Food
UV	Ultraviolet
WHO	World Health Organisation